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(54) Title: RAPID, SELF-PERFORMING TSH IMMUNOASSAY

(57) Abstract

An immuno-chromatographic TSH assay has now been developed which produces a visible result within minutes of sample application. The assay comprises a labeled antibody in a mobile phase and a second antibody in a stationary phase. The antibodies are chosen such that they are preferably non-competitive; i.e., they bind to different epitopes on the TSH and thus form a "sandwich" complex when both antibodies are bound to the TSH. In operation, sample TSH reacts with the mobile antibody and then both are transported to the bound antibody and form a "sandwich" therewith. Over a short time, if there is sufficient TSH in the sample, the concentration of TSH increases until it produces a visible spot in the area of the stationary phase. The TSH assay of the present invention may be used in screening for primary hypothyroidism in neonates and adults. It may also be used in combination with TRH administration to screen for hyperthyroidism.

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TITLE OF THE INVENTION

RAPID, SELF-PERFORMING TSH IMMUNOASSAY

BACKGROUND OF THE INVENTION

Thyroid stimulating hormone (TSH, thyrotropin) is a 28 kD glycoprotein composed of covalently bound alpha and beta subunits which are produced by the anterior pituitary gland in response to hypothalamic stimulation. TSH regulates the production of the thyroid hormones triiodothyronine (T3) and thyroxine (T4). These hormones have various physiologic effects; primarily, they increase protein synthesis and they increase oxygen consumption in all organs and tissues. Primary hypothyroidism is the decreased secretion of T4, which is part of a negative feedback system that controls TSH production. Reduction in T4 production results in elevated levels of circulating TSH. Primary hypothyroidism is associated with various disease states. Assaying for TSH is a primary screen in the diagnosis of these conditions. Normal TSH levels in neonates (i.e., persons up to 4 days *post partum*) is in the range 1-17.5±2.5 μIU(International Units)/mL. In adults (i.e., all persons older than neonates) the range is 0.3-5 μIU/mL. At present, fluorometric, chemoluminescent assays, enzyme-linked immunoassays, and radioimmunoassays (RIAs) can measure serum TSH. However, these require expensive and time-consuming equipment and an infrastructure comprising centralized laboratories and skilled technicians to perform the assays. Congenital hypothyroidism, if untreated, can result in irreversible cretinism by six weeks *post partum*. Centralized laboratories in the industrialized nations annually screen about 12 million neonates for congenital hypothyroidism. Assay results from an on-site laboratory take about 24 hours to obtain; from centralized laboratories, up to about three weeks. Whereas these results are generally available in the industrialized nations, in other parts of the world delays in obtaining assay results and

their unreliability can have serious consequences. For example, an estimated 800 million people live in endemic goiter regions, resulting in the birth of about 3.15 million cretins annually. Early, mass screening followed by treatment with synthetic T_4, would prevent most of these cases. Self-performing, rapid immunoassays using labeled and bound antibody pairs to detect various analytes have been described; e.g., UK Patent GB 2,204,398, May et al., and EP 42,755, Davis et al. However, none have been described for intact human TSH.

TRH (thyrotropin releasing hormone), a cyclic tripeptide hormone secreted by the hypothalamus, stimulates the release of TSH from the anterior pituitary gland. TRH is commonly administered, i.v. or orally, to patients as a diagnostic tool when there is a question of thyroid disease, since the patient's TSH response to TRH can be measured and this information used to, for example, exclude a diagnosis of hyperthyroidism. The normal response to TRH administration is a prompt rise in serum TSH levels. When 100-500 µg TRH is administered i.v., TSH levels normally rise over a period of 1-180 minutes 5-35 µIU/mL, with a peak value in 20-30 minutes, maximally at 22±2 minutes. A serum TSH rise in this time period greater than 5 µIU/mL after the administration of TRH rules out hyperthyroidism. Thus, a device which can indicate whether serum TSH levels exceed 5 µIU/mL can be used to rule out a diagnosis of hyperthyroidism when used in conjunction with TRH administration.

There is, therefore, a need for a reliable, self-performing screening assay for TSH which can be performed without the need for specialized equipment or highly trained personnel.

SUMMARY OF THE INVENTION

An immunochromatographic, self-performing TSH assay has now been developed which produces a visible result within minutes of sample application. The assay comprises a labeled, preferably non-isotopically labeled, antibody in a mobile phase and a second, preferably

a different, antibody in a stationary phase. The antibodies are, preferably, chosen such that they are non-competitive; i.e., they bind to different epitopes of, preferably intact, TSH and thus form a "sandwich" complex when both antibodies are bound to the TSH. In operation, TSH from the test sample reacts with the mobile antibody and then both are transported to the bound antibody and form a "sandwich" therewith. Over a short time, if there is sufficient TSH in the sample, the concentration of TSH to which labeled antibody is attached increases until it produces a visible spot in the area of the stationary phase. The TSH assay of the present invention may be used in screening for primary hypothyroidism in neonates and adults. It can also be used in confirming a diagnosis of hypothyroidism or in monitoring the treatment of hypothyroidism. The assay may also be used in monitoring the treatment of thyroid cancer, as hypothyroidism is required for treatment with radioactive iodine. When used in conjunction with TRH administration, the assay may be used to exclude a diagnosis of hyperthyroidism.

DETAILED DESCRIPTION OF THE INVENTION

It is an object of the present invention to provide a TSH assay which produces either a quantitative or semi-quantitative result, is complete within about 5-10 minutes of sample application, can be performed by an unskilled person, and requires no additional apparatus other than an assay kit.

The kit comprises a substrate divided into four, optionally five, regions. The first region is a reservoir for accepting the aqueous test sample, typically drops of whole blood, although serum or plasma may also be used. Next is a region coated with labeled antibody which has been selected for its high affinity (i.e., $K_{eq} \geq 10^6$) to an epitope of human TSH, preferably intact TSH. Preferably, this is a colored label such as a colloidal dye or colloidal metal, such as gold, which in concentrated amounts can be seen by the naked eye without the need for instrumentation such as a scintillation counter or spectrophotometer. The antibody and label are not bound to the substrate. Rather, they are coated onto it but are free to migrate when exposed to an aqueous liquid

such as the fluid from the test sample. The third region, the detection zone, contains a second, typically unlabeled, antibody, which is bound to the substrate. This antibody is also selected for its high affinity to human TSH, preferably intact TSH, but, preferably, differs from the first in that it is selective for a different TSH epitope and thus is not competitive with the first antibody. The antibodies used in both regions are preferably monoclonal antibodies. The fourth region acts as a control. This region contains a control reagent that either 1) binds to the labeled antibody, such as TSH or antibody to the labeled antibody; or 2) changes color when hydrated, such as anhydrous copper sulfate. The last region is a sink which absorbs any liquid which has traveled from the reservoir through regions 2-4. Although described as five regions, the substrate may be one continuous material. Alternatively, the regions may be of different materials; however; each region must be in intimate contact with any adjacent region such that fluid flows, typically by capillary action, unhindered from one region to the next. Each of these regions will be further described below.

The antibodies in the second and third regions are chosen for their selectivity to different TSH epitopes. Thus, either one or the other may be selected for its affinity to the α, β, or α,β subunits, with the proviso that at least one has an affinity to β. Additionally, both may have an affinity to β, although this may result in lower assay sensitivity. Preferably, one antibody is selective for β subunits and the other for α,β subunits.

Various physical configurations can be envisioned to achieve the objective of the invention. For example, the labeled antibody may be coated on the inside surface of a cup or well which acts as the sample reservoir. The detection zone, the control, and the sink may be on a stick or flat substrate which is dipped into said well after a sample has been placed in the well. In operation the detection zone is below the control but above the sample when the stick is lowered into the sample. The labeled antibody may be added substantially concomitantly with the sample to the reservoir instead of being precoated on the substrate.

Preferable, however, is a configuration which comprises a rigid, moisture-impervious solid sheath, generally flat and rectangular in shape, which contains and rigidly holds a substrate in the form of a strip or sheet made of a liquid-conductive material, said material coated with labeled antibody, the bound second antibody, and the control reagent. The sheath is preferably opaque. The upper surface of the sheath contains at least one hole, located over the reservoir end of the substrate, which is used to apply the test sample to the reservoir, and one or two viewing portals, which may either be holes or clear plastic areas, along the center line of said upper surface. The viewing portals are located over the detection zone and the control. If the portals are small, then two portals are used. Alternatively, an elongated single portal which provides visual access to both regions may be used. The upper surface of the sheath may be marked to indicate the detection and control zones. The upper surface of the sheath may also be marked along the detection portal to indicate a range of concentrations when the assay is quantitative. Since the reagents and substrate should be kept dry until used, a thin, transparent, moisture-impermeable material may be sandwiched between the substrate and the upper surface of the sheath and the hole over the reservoir may be covered with a removable tight fitting cap. A desiccant such as silica gel may also be contained within the sheath. Alternatively, the sheath may be designed to be less moisture-impermeable if the entire sheath is sealed, together with an optional desiccant, in a moisture-impermeable (e.g., plastic) wrap at the time of manufacture. Devices of this type are commercially available (e.g., Veda Lab, Alencon, France; Solar Care, Bethlehem, PA) and have been described in, e.g., UK Patent GB 2,204,398, which is incorporated herein by reference. Commercially available sheaths are about 7 x 3 x 0.4 cm and contain substrates about 6 x 0.8 cm. Such devices are readily adaptable for use in the present invention.

The reservoir can be made from any material capable of absorbing liquid rapidly, preferably within a few seconds of sample application. If commercially available sheaths and substrates as described above are used, the sample size is about four drops (100 μL) from a pipette. The test sample may also be collected on a fibrous material, such as a cotton swab which is then depressed onto the

reservoir. The hole over the reservoir may also be fitted with a scoop- or funnel-shaped cap which can be used to collect blood, as from a finger prick, and convey it to the reservoir. Polypropylene, polyethylene, polyvinylidene fluoride, ethylene vinylacetate, acrylonitrile, polytetrafluoro-ethylene, nylon, paper or other cellulosics, such as nitrocellulose, and similar materials may be used. To enhance liquid uptake, these materials may be treated with surfactants to reduce hydrophobicity. Since the sample to be tested may be whole blood, the reservoir also contains a filter which traps red and white blood corpuscles, thus preventing them from migrating into the detection zone.

The substrate may be made of the same materials as the reservoir. The substrate is preferably nitrocellulose with a pore size of about 1-12 microns, preferably at least 5 microns, and more preferably 8-12 microns. Nitrocellulose is advantageous in that antibody may be bound to it without prior chemical treatment. When paper is used for the substrate, binding of the antibody is done using cyanobromide, carbonyldiimidazole, or tresyl chloride. To give it some rigidity, the substrate may be adhered to a backing, preferably made of a thin plastic material. In regions 2-4, the substrate may be sandwiched between two pieces of plastic backing for protection of the reagents thereon. Since the assay comprises a timed reaction, the substrate material is chosen such that liquid migrates through it at a maximum flow rate of about 0.5-0.75 cm/min when the device is in a horizontal position. Further, the spacing between regions 2-4 must be selected such that there is sufficient time for uptake of the labeled antibody in region 2 by the migrating liquid and reaction with the bound antibody in the detection zone. Another timing requirement is that the control should indicate that the assay is complete in 5-10 minutes. Migration rate can be controlled by application to the substrate of surfactants either alone or in combination with viscosity modifiers such as sugars (e.g., sucrose or lactose), cellulosics, or gums (plant or microbial). Migration of sample fluid is via capillary action, which can be enhanced by osmotic pressure or gravity. Osmotic pressure is applied by the oncotic pressure of plasma proteins. Gravity can be applied by either constructing the assay kit such that the reservoir is higher than the distal end of the substrate

when the kit is placed on a horizontal surface or by instructing the user to position the reservoir higher than the distal end after application of the test sample.

The second region of the substrate contains the labeled antibody. Since this antibody must be mobile when exposed to the test sample, it is preferable that it be applied substantially as a surface coating rather than be impregnated into the substrate. This can be achieved by first glazing the second region with compound such as an aqueous solution of sugar, cellulosic, or gum, drying said glaze, and then applying the antibody to the glaze. The antibody applied to the second region is one of a pair of antibodies produced by methods well known in the art which are selected for their high affinity to, preferably intact, TSH and also because, preferably, they bind to different epitopes on, preferably intact, TSH; i.e., their binding is non-competitive. The antibodies should also be selected such that they exhibit little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), or follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins. These selections may be made using known protocols to determine cross reactivity or interference with samples containing known amounts of the hormones, proteins, etc. being tested against. The mobile antibody is bound to a label as by covalent or hydrophobic binding such that both antibody and label migrate together into the detection zone. The label may be any readily detectable material. Preferably, it is detectable by the naked eye, such as a colloidal dye, colloidal metal, or colored polymer particles. However, labels which are detectable by use of optical filters, by stimulation as by UV or fluorescent light, or by the addition of developing reagents may also be used. These latter, known as indirect labels, are less desirable since they require the addition of the developing reagents, typically enzymes such as alkaline phosphatase, to complete the assay. The preferred label is colloidal gold. Labels of this sort are well known in the art. Colloidal metals are described in EP 7654. Colloidal dyes are described in EP 32270. Colored polymer particles are described in UK 2,204,398.

The third region of the substrate, the detection zone, contains a bound antibody which complements the mobile antibody, as described above. Unlike the application of antibody to the surface of the substrate in second region, it is preferable to impregnate the substrate with antibody in the detection zone to maximize the amount of TSH captured during the assay. The amount of antibody applied in this region determines the intensity of the signal produced during the assay since the labeled antibody in the second region is applied in excess. This amount is determined by calibration against a reference standard containing a known amount of TSH.

Both quantitative and semi-quantitative signals can be produced. If a homogenous, narrow band of unlabeled antibody is applied across the width of the substrate, it will produce a uniform color band when exposed to labeled TSH-antibody complex from region 2. Thus, a semi-quantitative assay kit can be produced which will detect hypothyroidism in adults or rule out a diagnosis of hyperthyroidism by applying enough antibody in the detection zone to produce a signal only if the test sample contains greater than 5 μIU/mL of TSH. A semi-quantitative kit can also be produced which will detect hypothyroidism in neonates by applying enough antibody in the detection zone to produce a signal only if the test sample contains greater than 15, preferably greater than 20, μIU/mL of TSH.

If the unlabeled antibody is applied with a calibrated concentration gradient, then a series of signals will be produced during the assay. Alternatively, a homogenous band of unlabeled antibody may be applied along the length of an elongated detection zone (e.g., 4 cm of a 6 cm long substrate). Either of these configurations will produce a signal which can be interpreted quantitatively if the portion of the sheath adjacent to the portal overlying the detection zone is calibrated to indicate different levels of TSH, for example, in the ranges 0-25 or 0-40 μIU/mL.

After impregnation with unlabeled antibody, the substrate is dried and then treated with a blocking agent to block any free binding sites not already bound to antibody. The blocking agent may be one or

more of protein, such as bovine serum albumen or casein, polyvinyl alcohol, or ethanolamine. Alternatively, the blocking agent can be applied in excess to the substrate between the second and third regions. During the assay the excess agent will be carried along by the fluid from the test sample and block free binding sites in the detection zone.

The fourth, or control, region is designed to indicate that the assay device is working properly. This is achieved by any of several means. For example, the substrate may be treated with mono- or polyclonal IgG from a non-mouse species (e.g., avian, bovine, equine, etc.), such as unlabeled goat (anti-mouse IgG) antibody, which will react with the labeled murine monoclonal antibody. Preferably, the control reagent specifically binds with high affinity to the immunoglobulin of the species used to produce the labeled antibodies. Alternatively, the substrate may be treated with TSH, which will react with the labeled antibody. Such reactive materials are preferred, since in addition to merely indicating that fluid from the test sample has traversed regions 2-3, they can minimize false positives which can result from mis-interpreting weak signals in the detection zone caused by subcritical amounts of TSH being present in the test sample. The user would be instructed to compare the signals in the detection and control zones. The absence of a control signal would indicate a failed assay; i.e., one which is not reliable and should be repeated. Instead of reactive materials, the control region may be treated with a substance which will change color when moistened, such as anhydrous copper sulfate. Such a signal generated in the control region is not part of the assay per se but is a positive control indicating that fluid from the test sample has traversed regions 2-3. The control reagents are preferably bound to the substrate.

It is preferred that there be a continuous flow of liquid from the reservoir and through each of regions 2-4. Therefore, a sink at the distal end of the substrate is desirable. The sink may be made of any highly absorbent material and may be either an extension of the substrate itself or some other material in contact with the substrate. Thus, a nitrocellulose substrate may have attached to it a sink of chromatography paper. The sink should be of sufficient mass that it can

continue to absorb whatever liquid reaches it from the reservoir during the five-ten minute period of the assay, thus assuring continued migration of the test sample across the detection zone.

It is understood that the assay described herein is dynamic, i.e., after application of the sample there is constant movement of liquid and components thereof through the various regions of the kit. Some components may remain in one region, travel to an adjacent region, be partially or completely depleted by reaction with another component, etc. This dynamism is inherent in the embodiments of the invention described below. Thus, in step 2 of the assay, "the sample" which migrates from the first region is clearly meant to describe that portion of the applied sample of step 1 which was not retained in the first region. (The retained portion may include some liquid and red and white blood corpuscles.) Similarly, only a portion of the labeled antibody is complexed in region 2 (the labeled antibody having been applied in excess), so that a portion of labeled antibody may also migrate to region 4.

One embodiment of the invention is a method of screening for primary hypothyroidism or ruling out a diagnosis of hyperthyroidism which comprises reacting a sample of human blood with labeled antibody with a high affinity to an epitope of, preferably intact, human TSH in a mobile phase to form a TSH-antibody complex and then reacting said complex with an immobilized antibody with a high affinity to an epitope of, preferably intact, human TSH to form a detectable antibody-TSH-antibody complex.

Another embodiment of the invention is an immunochromatographic, self-performing assay for measuring, preferably intact, human TSH in the range 0-40 μIU/mL in an aqueous sample which comprises:

1) applying the sample to a reservoir of a kit which comprises a substrate divided into four, optionally five, regions the first of which is a reservoir for accepting said sample;

2) allowing said sample to migrate from said first region through a second region which is a region coated with unbound, labeled,

preferably monoclonal, antibody which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, whereby labeled antibody-TSH complex is formed;

3) allowing said sample and labeled antibody-TSH complex to migrate from said second region through a third region containing a second, preferably monoclonal, antibody, bound to the substrate, which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, with the proviso that at least one of said antibodies has a high affinity to β subunits, whereby a labeled antibody-TSH-antibody complex is formed;

4) allowing said sample and said labeled antibody to migrate from said third region through a fourth region which contains a control reagent that either 1) binds to the labeled antibody or 2) changes color when hydrated; and

5) optionally, allowing said sample to migrate from said fourth region to a final region which is a sink which absorbs any liquid which has migrated along said substrate from said reservoir; and

6) visually detecting said labeled antibody-TSH-antibody complex in said third region within 5-10 minutes of applying said sample to the reservoir.

Another embodiment of the invention is a method of screening for primary hypothyroidism, confirming a diagnosis of hypothyroidism, monitoring treatment of hypothyroidism, monitoring treatment of thyroid cancer, or ruling out a diagnosis of hyperthyroidism which comprises:

1) applying a sample of human blood to a reservoir of a kit which comprises a substrate divided into four, optionally five, regions the first of which is a reservoir for accepting said sample of human blood;

2) allowing said sample to migrate from said first region through a second region which is a region coated with unbound, labeled, preferably monoclonal, antibody which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, whereby labeled antibody-TSH complex is formed;

3) allowing said sample and labeled antibody-TSH complex to migrate from said second region through a third region containing a second, preferably monoclonal, antibody, bound to the substrate, which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, with the proviso that at least one of said antibodies has a high affinity to β subunits, whereby a labeled antibody-TSH-antibody complex is formed;

4) allowing said sample and said labeled antibody to migrate from said third region through a fourth region which contains a control reagent that either 1) binds to the labeled antibody or 2) changes color when hydrated; and

5) optionally, allowing said sample to migrate from said fourth region to a final region which is a sink which absorbs any liquid which has migrated along said substrate from said reservoir; and

6) visually detecting said labeled antibody-TSH-antibody complex in said third region within 5-10 minutes of applying said sample to the reservoir.

In the embodiments described above it is preferred that the unbound antibody is non-isotopically labeled. It is also preferred that the sample is human blood. It is also preferred that the antibodies in regions 2 and 3 are different from each other and monoclonal. It is also preferred that one antibody is selective for β subunits and the other for α,β subunits of TSH, preferably intact TSH. It is also preferred that the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 5 μIU/mL, or

greater than 15 μIU/mL, or greater than 20 μIU/mL, or in the range 0-25 μIU/mL, or in the range 0-40 μIU/mL.

Another embodiment of the invention is a method as described above wherein the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 5 μIU/mL

Another embodiment of the invention is a method as described above wherein the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 15 μIU/mL, or greater than 20 μIU/mL

Another embodiment of the invention is a method as described above wherein the antibody-TSH-antibody complex is detectable when the concentration of TSH in the sample is in the range 0-40, preferably 0-25 μIU/mL.

Another embodiment of the invention is an improved method for ruling out the diagnosis of hyperthyroidism in an adult person which comprises:

a) administering to said person 100-500 μg TRH orally or intravenously;

b) drawing a blood sample from said person 1-180 min., preferably 20-30 min., more preferably 22±2 min. after said TRH administration; and

c) determining whether the serum TSH level of said blood sample exceeds 5 μIU/mL

wherein the determination comprises:

1) applying the sample of said person's blood drawn after TRH administration to a reservoir of a kit which comprises a substrate divided into four, optionally five, regions the first of which is a reservoir for accepting said sample of human blood;

2) allowing said sample to migrate from said first region through a second region which is a region coated with unbound, labeled,

preferably monoclonal, antibody which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, whereby labeled antibody-TSH complex is formed;

3) allowing said sample and labeled antibody-TSH complex to migrate from said second region through a third region containing a second, preferably monoclonal, antibody, bound to the substrate, which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, with the proviso that at least one of said antibodies has a high affinity to β subunits, whereby a labeled antibody-TSH-antibody complex is formed;

4) allowing said sample and said labeled antibody to migrate from said third region through a fourth region which contains a control reagent that either 1) binds to the labeled antibody or 2) changes color when hydrated; and

5) optionally, allowing said sample to migrate from said fourth region to a final region which is a sink which absorbs any liquid which has migrated along said substrate from said reservoir; and

6) visually detecting said labeled antibody-TSH-antibody complex in said third region within 5-10 minutes of applying said sample to the reservoir.

In the embodiment described above it is preferred that the unbound antibody is non-isotopically labeled. It is also preferred that the antibodies in regions 2 and 3 are different from each other and monoclonal. It is also preferred that one antibody is selective for β subunits and the other for α,β subunits of TSH, preferably intact TSH.

Another embodiment of the invention is a kit for screening for primary hypothyroidism which comprises a rigid, moisture-impervious solid sheath containing therein a substrate divided into four, optionally five, regions the first of which is a reservoir for accepting a

sample of human blood, the second of which is a region coated with unbound, labeled antibody which has been selected for its high affinity to an epitope of, preferably intact, human TSH, the third of which is a region containing a second antibody, bound to the substrate, which has been selected for its high affinity to an epitope of, preferably intact, human TSH, the fourth of which is optional and contains a control reagent that either 1) binds to the labeled antibody or 2) changes color when hydrated, and the last of which is optional and is a sink which absorbs any liquid which has traveled from the first region.

In the embodiment described above it is preferred that the unbound antibody is non-isotopically labeled and monoclonal. It is also preferred that the antibodies in regions 2 and 3 are different from each other and monoclonal. It is also preferred that one antibody is selective for β subunits and the other for α,β subunits of,.preferably intact, TSH. It is also preferred that the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 5 μIU/mL, or greater than 15 μIU/mL, or greater than 20 μIU/mL, or in the range 0-25 μIU/mL, or in the range 0-40 μIU/mL.

Another embodiment of the invention is a kit for measuring, preferably intact, human TSH in the range 0-40 μIU/mL in an aqueous sample which comprises:

1) applying the sample to a reservoir of a kit which comprises a substrate divided into four, optionally five, regions the first of which is a reservoir for accepting said sample;

2) allowing said sample to migrate from said first region through a second region which is a region coated with unbound, labeled, preferably monoclonal, antibody which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, whereby labeled antibody-TSH complex is formed;

3) allowing said sample and labeled antibody-TSH complex to migrate from said second region through a third region containing a second, preferably monoclonal, antibody, bound to the substrate, which

has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, with the proviso that at least one of said antibodies has a high affinity to β subunits, whereby a labeled antibody-TSH-antibody complex is formed;

4) allowing said sample and said labeled antibody to migrate from said third region through a fourth region which contains a control reagent that either 1) binds to the labeled antibody or 2) changes color when hydrated; and

5) optionally, allowing said sample to migrate from said fourth region to a final region which is a sink which absorbs any liquid which has migrated along said substrate from said reservoir; and

6) visually detecting said labeled antibody-TSH-antibody complex in said third region within 5-10 minutes of applying said sample to the reservoir.

In the embodiment described above it is preferred that the unbound antibody is non-isotopically labeled. It is also preferred that the sample is human blood. It is also preferred that the antibodies in regions 2 and 3 are different from each other and monoclonal. It is also preferred that one antibody is selective for β subunits and the other for α,β subunits of, preferably intact, TSH. It is also preferred that the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 5 μIU/mL, or greater than 15 μIU/mL, or greater than 20 μIU/mL, or in the range 0-25 μIU/mL, or in the range 0-40 μIU/mL.

The invention is further defined by reference to the following examples, which are intended to be illustrative and not limiting.

EXAMPLE 1

Kit Assembly

1) Unlabeled anti-TSH antibody (one of a pair of murine monoclonal antibodies directed against distinct epitopes of intact human

TSH) is deposited in a band across the width of nitrocellulose paper (8-12 μ pore size with plastic backing). Antibody may be obtained from Kara Biologicals, Stanton, N.J. Nitrocellulose paper may be obtained from Schleicher and Schuell, Keene, N.H. After application of the antibody, the nitrocellulose paper is dried.

2) Deposit a band of unlabeled goat (anti-mouse IgG) antibody approximately 1 cm away from the antibody band. After application of the IgG, the nitrocellulose paper is again dried.

3) Treat the nitrocellulose paper with bovine serum albumin, casein, polyvinyl alcohol, or ethanolamine to block any non-specific binding sites thereon. After application of the blocking agent, the nitrocellulose paper is again dried.

4) Overlay a pad of absorbent material onto the nitrocellulose paper ~1 cm away from the antibody band of Step 1 and at the opposite end from the IgG band of Step 2.

5) On said overlay deposit the second of the pair of murine monoclonal antibodies, labeled with colloidal gold (Kara Biologicals). After application of the labeled antibody, the nitrocellulose paper is again dried.

6) The nitrocellulose paper is cut into strips approx. 6 x 0.8 cm such that the absorbent pad is at one end of each strip.

7) A strip from step 6 is placed in the bottom half of a 7 x 3 x 0.4 cm plastic sheath. The absorbent pad is overlaid with a blood elements filter (Millipore Corp., Bedford, Mass.). The distal end of the strip beyond the IgG band is overlaid with a piece of bibulous paper (Schleicher and Schuell). A desiccant such as silica gel is also placed in the bottom half of the sheath below the strip.

8) The top half of the sheath is affixed to the bottom half such that a portal suitable for adding test sample overlies the blood elements filter and a portal suitable for viewing overlies the unlabeled antibody band and the IgG band.

9) Optionally, the top half of the sheath contains means for collecting capillary blood from a puncture wound, as described in U.S.P. 4,397,318, 4,653,512, and 4,646,753. Additionally, parallel to the viewing portal may be inscribed a scale for determining the amount of TSH which becomes bounded to the unlabeled antibody. The interior of the top half of the sheath may also comprise one or more crimping means which constrain the blood elements filter, the absorbent pad, and the bibulous paper against the elements immediately below them. The amounts of antibodies applied in Steps 1, 2, and 5 are empirically determined by applying known protocols to one or more samples with known concentrations of TSH and based on the desired range or desired cut-off of assay sensitivity.

EXAMPLE 2
Diagnosis of Hyperthyroidism Following TRH Administration

Three patients with enlarged thyroid glands and questionable hyperthyroidism had a serum sample drawn. Immediately thereafter, 250 µg TRH was administered to each patient *i.v.* Second serum samples were drawn 22 min. following TRH administration. The TSH levels in each pair of samples were measured using both a third generation TSH assay and a rapid TSH assay as in Example 1 calibrated for a 5 µIU/mL cutoff. In each patient the basal (i.e., t = 0) TSH value (third generation assay) was low and the incremental rise (i.e., TSH t_{22}-t_0) was less than 1 µIU/mL, confirming the diagnosis of hyperthyroidism. Using the rapid TSH assay of the invention, none of the TSH concentrations exceeded 5 µIU/mL (i.e., only lines in the control regions were detected), thus, also confirming the diagnosis of hyperthyroidism and also demonstrating the equivalence of a third generation TSH assay and a rapid TSH assay of the invention for the diagnosis of hyperthyroidism following TRH administration.

Each of the patents and patent applications recited herein is hereby incorporated by reference.

WHAT IS CLAIMED IS:

1. A method of screening for primary hypothyroidism, confirming a diagnosis of hypothyroidism, monitoring the treatment of hypothyroidism, monitoring the treatment of thyroid cancer, or ruling out a diagnosis of hyperthyroidism which comprises 1) reacting a sample of human blood with labeled antibody with a high affinity to an epitope of, preferably intact, human TSH in a mobile phase to form a TSH-antibody complex and 2) reacting said complex with an antibody with a high affinity to an epitope of, preferably intact, human TSH in a stationary phase to form a detectable antibody-TSH-antibody complex within 5 to 10 minutes of reacting said sample with said labeled antibody, with the proviso that at least one antibody has an affinity to TSH β subunit.

2. A quantitative method of Claim 1 wherein the antibody-TSH-antibody complex is detectable when the concentration of TSH in the sample is in the range 0-25 μIU/mL.

3. A semi-quantitative method of Claim 1 of screening for primary hypothyroidism in adults wherein the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 5 μIU/mL

4. A semi-quantitative method of Claim 1 of screening for primary hypothyroidism in neonates wherein the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 15 μIU/mL

5. A semi-quantitative method of Claim 1 of screening for primary hypothyroidism in neonates wherein the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 20 μIU/mL

6. A method of Claim 1 wherein the label is a colloidal dye, a colloidal metal, or a colored polymer particle and the antibodies are monoclonal antibodies.

7. A method of screening for primary hypothyroidism, confirming a diagnosis of hypothyroidism, monitoring treatment of hypothyroidism, monitoring treatment of thyroid cancer, or ruling out a diagnosis of hyperthyroidism which comprises:

1) applying a sample of human blood to a reservoir of a kit which comprises a substrate divided into four, optionally five, regions the first of which is a reservoir for accepting said sample of human blood;

2) allowing said sample to migrate from said first region through a second region which is a region coated with unbound, labeled antibody which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, whereby labeled antibody-TSH complex is formed;

3) allowing said sample and labeled antibody-TSH complex to migrate from said second region through a third region containing a second antibody, bound to the substrate, which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, with the proviso that at least one of said antibodies has a high affinity to β subunits, whereby a labeled antibody-TSH-antibody complex is formed;

4) allowing said sample and said labeled antibody to migrate from said third region through a fourth region which contains a control reagent that either 1) binds to the labeled antibody or 2) changes color when hydrated; and

5) optionally, allowing said sample to migrate from said fourth region to a final region which is a sink which absorbs any liquid which has migrated along said substrate from said reservoir; and

6) visually detecting said labeled antibody-TSH-antibody complex in said third region within 5-10 minutes of applying said sample to the reservoir.

8. A method of Claim 7 wherein the substrate is nitrocellulose with a pore size of about 1-12 microns; the label is a colloidal dye, a colloidal metal, or a colored polymer particle; and the antibodies are monoclonal antibodies.

9. A method of Claim 7 wherein the unbound antibody is non-isotopically labeled, the antibodies in regions 2 and 3 are different from each other and monoclonal, and one said antibody is selective for the β subunit of intact TSH.

10. A method of Claim 7 of screening for primary hypothyroidism in adults wherein the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 5 μIU/mL.

11. A method of Claim 7 of screening for primary hypothyroidism in neonates wherein the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 15 μIU/mL.

12. A method of Claim 7 of screening for primary hypothyroidism in neonates wherein the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 20 μIU/mL.

13. A quantitative method of Claim 7 wherein the antibody-TSH-antibody complex is detectable when the concentration of TSH in the sample is in the range 0-25 μIU/mL.

14. An immunochromatographic, self-performing assay for measuring, preferably intact, human TSH in the range 0-40 μIU/mL in an aqueous sample which comprises:

1) applying the sample to a reservoir of a kit which comprises a substrate divided into four, optionally five, regions the first of which is a reservoir for accepting said sample;

2) allowing said sample to migrate from said first region through a second region which is a region coated with unbound, labeled antibody which has been selected for its high affinity to an epitope of,

preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, whereby labeled antibody-TSH complex is formed;

3) allowing said sample and labeled antibody-TSH complex to migrate from said second region through a third region containing a second antibody, bound to the substrate, which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, with the proviso that at least one of said antibodies has a high affinity to β subunits, whereby a labeled antibody-TSH-antibody complex is formed;

4) allowing said sample and said labeled antibody to migrate from said third region through a fourth region which contains a control reagent that either 1) binds to the labeled antibody or 2) changes color when hydrated; and

5) optionally, allowing said sample to migrate from said fourth region to a final region which is a sink which absorbs any liquid which has migrated along said substrate from said reservoir; and

6) visually detecting said labeled antibody-TSH-antibody complex in said third region within 5-10 minutes of applying said sample to the reservoir.

15. A method of Claim 14 wherein the substrate is nitrocellulose with a pore size of about 1-12 microns; the label is a colloidal dye, a colloidal metal, or a colored polymer particle; and the antibodies are monoclonal antibodies.

16. A method of Claim 14 wherein the unbound antibody is non-isotopically labeled, the antibodies in regions 2 and 3 are different from each other and monoclonal, and one said antibody is selective for the β subunit of intact TSH.

17. A method of Claim 14 wherein the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 5 μIU/mL.

18.. A method of Claim 14 wherein the antibody-TSH-antibody complex is detectable only when the concentration of TSH in the sample is greater than 20 μIU/mL.

19. A method of Claim 14 wherein the antibody-TSH-antibody complex is detectable when the concentration of TSH in the sample is in the range 0-25 μIU/mL.

20. An improved method for ruling out the diagnosis of hyperthyroidism in an adult person which comprises:

a) administering to said person 100-500 μg TRH orally or intravenously;

b) drawing a blood sample from said person 1-180 min. after said TRH administration; and

c) determining whether the serum TSH level of said blood sample exceeds 5 μIU/mL

wherein the determination comprises:

1) applying the sample of said person's blood drawn after TRH administration to a reservoir of a kit which comprises a substrate divided into four, optionally five, regions the first of which is a reservoir for accepting said sample of human blood;

2) allowing said sample to migrate from said first region through a second region which is a region coated with unbound, labeled antibody which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, whereby labeled antibody-TSH complex is formed;

3) allowing said sample and labeled antibody-TSH complex to migrate from said second region through a third region containing a second antibody, bound to the substrate, which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, with the proviso that at least one of said antibodies has a high affinity to β subunits, whereby a labeled antibody-TSH-antibody complex is formed;

4) allowing said sample and said labeled antibody to migrate from said third region through a fourth region which contains a control reagent that either 1) binds to the labeled antibody or 2) changes color when hydrated; and

5) optionally, allowing said sample to migrate from said fourth region to a final region which is a sink which absorbs any liquid which has migrated along said substrate from said reservoir; and

6) visually detecting said labeled antibody-TSH-antibody complex in said third region within 5-10 minutes of applying said sample to the reservoir.

21 A method of Claim 20 wherein the unbound antibody is non-isotopically labeled, the antibodies in regions 2 and 3 are different from each other and monoclonal, one antibody is selective for β subunits and the other for α,β subunits of intact TSH, and the blood is drawn 20-30 min. after TRH administration.

22 A method of Claim 21 wherein the blood is drawn 22±2 min. after TRH administration.

23. A kit for screening for primary hypothyroidism, confirming a diagnosis of hypothyroidism, monitoring the treatment of hypothyroidism, monitoring the treatment of thyroid cancer, or ruling out a diagnosis of hyperthyroidism which comprises a rigid, moisture-impervious solid sheath containing therein a substrate divided into four, optionally five, regions:

the first of which is a reservoir for accepting a sample of human blood,

the second of which is a region coated with unbound, labeled antibody which has been selected for its high affinity to an epitope of, preferably intact, human TSH,

the third of which is a region containing a second antibody, bound to the substrate, which has been selected for its high affinity to an epitope of, preferably intact, human TSH,

the fourth of which contains a control reagent that either 1) binds to the labeled antibody or 2) changes color when hydrated, and

the last of which is optional and is a sink which absorbs any liquid which has migrated along said substrate from the reservoir.

24. A kit of Claim 23 wherein the unbound antibody is non-isotopically labeled, the antibodies in regions 2 and 3 are different from each other and monoclonal, and one said antibody is selective for the β subunit of intact TSH.

25. A kit of Claim 23 wherein the substrate is nitrocellulose with a pore size of about 1-12 microns; the label is a colloidal dye, a colloidal metal, or a colored polymer particle; and the antibodies are monoclonal antibodies.

26. A kit of Claim 23 wherein a antibody-TSH-antibody complex is detectable in said third region when the concentration of TSH in the blood sample is in the range 0-25 μIU/mL.

27. A kit of Claim 23 for screening for primary hypothyroidism or ruling out a diagnosis of hyperthyroidism in adults wherein a antibody-TSH-antibody complex is detectable in said third region only when the concentration of TSH in the blood sample is greater than 5 μIU/mL.

28. A kit of Claim 23 for screening for primary hypothyroidism in neonates wherein a antibody-TSH-antibody complex is detectable in said third region only when the concentration of TSH in the blood sample is greater than 15 μIU/mL.

29. A kit of Claim 23 for screening for primary hypothyroidism in neonates wherein a antibody-TSH-antibody complex is detectable in said third region only when the concentration of TSH in the blood sample is greater than 20 μIU/mL.

30. A kit for measuring, preferably intact, human TSH in the range 0-40 μIU/mL in an aqueous sample which comprises:

1) applying the sample to a reservoir of a kit which comprises a substrate divided into four, optionally five, regions the first of which is a reservoir for accepting said sample;

2) allowing said sample to migrate from said first region through a second region which is a region coated with unbound, labeled antibody which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, whereby labeled antibody-TSH complex is formed;

3) allowing said sample and labeled antibody-TSH complex to migrate from said second region through a third region containing a second antibody, bound to the substrate, which has been selected for its high affinity to an epitope of, preferably intact, human TSH and little to no cross reactivity with human chorionic gonadotropin (CG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and little to no interference from bilirubin, hemoglobin, triglycerides, or plasma proteins, with the proviso that at least one of said antibodies has a high affinity to β subunits, whereby a labeled antibody-TSH-antibody complex is formed;

4) allowing said sample and said labeled antibody to migrate from said third region through a fourth region which contains a control reagent that either 1) binds to the labeled antibody or 2) changes color when hydrated; and

5) optionally, allowing said sample to migrate from said fourth region to a final region which is a sink which absorbs any liquid which has migrated along said substrate from said reservoir; and

6) visually detecting said labeled antibody-TSH-antibody complex in said third region within 5-10 minutes of applying said sample to the reservoir.

31. A kit of Claim 30 wherein the unbound antibody is non-isotopically labeled; the antibodies in regions 2 and 3 are different from each other and monoclonal; one said antibody is selective for the β subunit of intact TSH; the substrate is nitrocellulose with a pore size of about 1-12 microns; the label is a colloidal dye, a colloidal metal, or a colored polymer particle; and the antibody-TSH-antibody complex is detectable in said third region when the concentration of TSH in the sample is in the range 0-25 μIU/mL.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02593

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GB, B, 2 204 398 (MAY et al.) 28 August 1991, page 5, line 26 to page 6, line 26; page 14, lines 9 to 13; page 17, lines 4 to 14; page 29, line 21 to page 30, line 19.	1-6
Y	US, A, 5,120,643 (CHING et al.) 09 June 1992, column 9, lines 3 to 10; column 16, line 60 to column 18, line 42.	1-6
Y	US, A, 5,266,497 (IMAI et al.) 30 November 1993, column 5, lines 4 to 11; column 9, lines 25 to 43.	1-6
Y	TUUMINEN et al. A rapid fluorometric enzyme immunoassay for the determination of neonatal TSH from blood spots. Clinica Chimica Acta. 31 October 1991, Vol. 202, No. 3, pages 167-178, especially pages 169-171.	1-6

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search 16 MAY 1996	Date of mailing of the international search report 31 MAY 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CAROL A. SPIEGEL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02593

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database Derwent Info Ltd. on Dialog, Dialog Information Services, Inc., (Palo Alto, CA, USA), No. 92-171661/21, KOKUSAI SHIYAKU KK 'Thyroid-stimulating hormone specific monoclonal antibody-for determination and purification of TSH by immunoassay with high sensitivity and specificity and no cross-reactivity to LH, HCG and FSH,' abstract, JP 4108397, July 1992.	1-6
A	US, A, 5,141,850 (COLE et al.) 25 August 1992, see entire document.	1-6
A	SOOS et al. Characterization of Monoclonal Antibodies Directed Against Human Thyroid Stimulating Hormone. Journal of Immunological Methods, 1982, Vol. 51, pages 57-68.	1-6
A	SPENCER et al. Serum TSH Measurement: A 1990 Status Report. Thyroid Today. October/November/December 1990, Vol. XIII, No. 4, pages 1-12.	1-6
A	Chem. abstr., Vol. 118, No. 15, 12 April 1993 (Columbus, OH, USA), page 99, column 2, the abstract No.118:139992y, TAKEOKA et al. 'Basic and clinical examination on the "BeriLux TSH" kit, a highly sensitive chemiluminescent immunoassay for TSH.' Takeoka. 1992, 27(4), 985-94 (Japan).	1-6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02593

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02593

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

G01N 33/53, 33/543, 33/545, 33/553

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.1, 7.9, 7.92, 7.94; 436/500, 518, 523, 525, 528, 531, 533, 535, 536, 538, 539, 548; 530/388.24

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

435/7.1, 7.9, 7.92, 7.94, 970; 436/500, 518, 523, 525, 528, 531, 533, 535, 536, 538, 539, 548, 810, 811, 817; 530/388.24

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

DIALOG, CAS, APS, JPO
search terms: tsh, thryotropin, thyroid stimulating hormone, monoclonal, test strip, dipstick, analytical element, colloidal, dye, label, marker

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This International Preliminary Examining Authority has found 3 inventions claimed in the International Application covered by the claims indicated below:

I.Claims 1-6, drawn to a process of screening thyroid function using a mobile, i.e. liquid phase, labeled anti-TSH antibody and a solid phase capture anti-TSH-antibody.
II.Claims 7-19 and 23-31, drawn to a process of screening thyroid function and an apparatus or means specifically designed for carrying out the process.
III.Claims 20-22, drawn to a process for diagnosing the occurrence of hyperthyroidism.

Inventions I-III each contain special and unique technical features. Invention II requires a specially designed apparatus for carrying out the independent process, i.e. an apparatus comprising a substrate divided into (1) a sample application reservoir, (2) a label region containing unbound, labeled antibody, (3) a capture region containing bound antibody, (4) an end-of-run control region, and optionally (5) an absorbent sink. Invention I does not require any specific apparatus or means for performing the independent two-site immunoassay process. Invention III requires a specific patient preparation,includingadministration of a challenging dose of thyroid releasing hormone and timing of sample withdrawals, to confirm the occurrence of hyperthyroidism. Thus, special unrelated technical features exist between Inventions I-III.

In the absence of any response from the applicant, this Authority will establish the International Search Report based on the main invention. The claims drawn to the main invention are as follows:
Claims 1-6.